Comparison of Oxidant and Antioxidant Status in the Aqueous Humour of Cataract Patients with and without Type 2 Diabetes Mellitus

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ABSTRACT

Objectives: To compare the total oxidant status (TOS), total antioxidant capacity (TAC), and oxidative stress index (OSI) in the aqueous humour of cataract patients with and without type 2 diabetes mellitus (T2DM).

Study Design: Cross-sectional analytical study.

Place and Duration of the Study: Department of Physiology, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan in collaboration with Armed Forces Institute of Ophthalmology, from 1st June 2022 to 31st January 2023.

Methodology: A total of 120 individuals were recruited and divided into 2 equal groups. Sample of aqueous humour was collected, and the total oxidant status, and total antioxidant status were measured by spectrophotometry. Blood samples were collected to measure the fasting glucose and glycosylated haemoglobin levels. Results were analysed by SPSS version 24 and p-value ≤0.05 was considered significant.

Results: Total oxidative stress and OSI were (55.57±5.46) *vs*. (33.75±4.28) = p (0.001), (32.79±9.23) *vs*. (13.28±3.02) = p (0.001) found significantly increased in aqueous humour of diabetic patients while TAC was reduced (1.86±0.41) *vs*. (2.62 ± 0.45) = p (0.001) compared to the non-diabetics. Fasting blood glucose (109.18±12.32 *vs.* 91.61±5.71 mg/dl, p=0.001) and glycosylated haemoglobin levels (6.46±0.85 *vs.*5.43±0.85, p=0.001) were also found significantly high in the diabetic group compared to the non-diabetics.

Conclusion: Increased levels of glucose and glycosylated haemoglobin increase oxidative stress in the blood of T2DM patients. This raises the total oxidative stress in the aqueous homor while lowering the TAC. This disturbed oxidant-antioxidant status in the aqueous humour of diabetic patients plays an important role in the development of cataract in T2DM patients compared to non-diabetics.

Key Words: *Cataract, Aqueous humour, Oxidative stress, Type 2 diabetes mellitus.*

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INTRODUCTION

Cataract is the reduction in the normal transparency of the crystalline lens due to an opacity. It is one of the most common reasons of early blindness across the world.[1] The pathogenesis of cataract in type 2 diabetes mellitus (T2DM) is considered to be a result of increased inflammation occurring in the anterior portion of the eye as well as in the blood vessels of many body organs. Hence, it is important to recognise the risk factors in order to prevent or start early treatment of visual loss due to the cataract.[2]

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A survey found that senile cataract had 15.3% overall rate of occurrence with age 30 years and older in 3 districts of the Punjab province of Pakistan, while it was 4.3% for all ages among 1,269 persons.[3] Gender difference in the incidence of age-related cataract in the Pakistani population was found to be higher in men (48%) compared to women (39%) with age of 70 years and above.[4]

The prevalence of cataract is three to four times higher in T2DM patients with age 65 years and below. Persistent hyperglycemia and poor metabolic control are considered as the main risk factors for cataract development.[5] The association between poor glycemic control and an increased incidence of cataract formation was also shown in the Beaver Dam Eye Study along with the faster progression of cataracts for T2DM patients.[6] Even though cataract formation is irreversible in older patients, there are bright chances of delay in the onset of cataract in young diabetics with better metabolic control.[5] Many studies have identified rise in various oxidative stress markers in the aqueous humour and blood of T2DM patients.[7,8] However,

compared to studying individual antioxidant or oxidant molecules as done in various studies, estimation of the total oxidant status (TOS) and total antioxidant capacity (TAC) of the aqueous humour could be a more practical and relevant approach.[9]

The developing countries suffer significant damage to health and consequently economic activities due to T2DM and cataract. The T2DM treatment is mostly insufficient and cataract surgery is many times out-of-the-reach in these countries.[10] Therefore, it is important to explore the link between the two diseases in order to minimise their adverse effects on the Pakistani population. The objective of this study was to compare the TOS, TAC, and OSI in the aqueous humour of cataract patients with and without T2DM.

METHODOLOGY

This cross-sectional analytical study was carried out from 1st June 2022 till 31st January 2023 at the Department of Physiology, Army Medical College, National University of Medical Sciences, Rawalpindi in collaboration with the Armed Forces Institute of Ophthalmology. After obtaining approval from the Institutional Ethical Review Committee (ERC/ID/206 dated 30 May 2022), 120 individuals were recruited and divided into two equal groups having 60 subjects in each. Group I consisted of 60 non-diabetic cataract patients while group II consisted of 60 cataract patients having T2DM. Sample size was calculated using online sample size calculator keeping confidence interval of 95%.

Patients of cataract (without any systemic illness), and patients with cataract as well as T2DM (group 2 only) including both genders of age 55-65 years were selected. Patients with any systemic illness for group I and those other than T2DM for group II were excluded along with patients with ophthalmic diseases other than cataract (like glaucoma, uveitis, prior ocular surgery, *etc*.) and those taking vitamin A, C, E supplements, non-steroidal anti-inflammatory drugs, smokers, and alcoholics/narcotics.

After giving the informed consent, all patients went through a complete ophthalmological checkup including a slit lamp biomicroscopic examination and a stereoscopic ocular fundus evaluation to rule out other ocular diseases. A comprehensive history and general physical examination were also carried out. The evaluation of cataract was done as per the Emery and Little classification depending upon the hardness of cataract.[11] On a separate proforma, data including the age, gender, duration of TD2M, FBG, eye disorders, and medications were endorsed.

At the start of the cataract surgery, prior to any conjunctival or intraocular intervention, 0.1–0.2 ml of the aqueous humour was extracted using a 26-gauge insulin syringe. The aqueous humour samples were taken to the Centre of Research Education and Applied Medicine Laboratory of Army Medical College / National University of Medical Sciences where they were stored at -80°C until biochemical analysis was carried out using ELISA.

The TAC was measured by enzyme-linked immunosorbent assay using Glory science kit (Cat AP001, Lot 1511470231). The assay is based on the oxidation of the ferrous ion-o-dianisidine complex by the oxidants in the sample to form ferric ion. This leads to the colour changes, and spectrophotometry is then used to measure the colour intensity. The aggregate of oxidant molecules is assessed and the results are expressed as millimolar Trolox equivalents per litre (mmol Eqv./L).

The TOS was measured using Elabscience kit (Cat E-BC-K802-M, Lot 1010876302) in the aqueous humour by enzyme-linked immunosorbent assay. The oxidants present in the sample oxidise ferrous to ferric ions in the acidic environment. The ferric ion binds highly with xylenol, which is yellowish-orange, to produce a blue-purple complex. When pH of the solution comes in the 2-3 range, its maximum absorption wavelength is near 590 nm and the depth of the colour is proportionate to the amount of oxidants present in the solution at a particular time. This permits the calculation of the total oxidation state of the solution indirectly.

After an overnight fast, 5ml blood was drawn from the antecubital vein to determine the FBG by hexokinase method using ADVIA 1800 clinical chemistry system (Randox glucose hexokinase, Randox laboratories, UK). The measurement of HbA1c in the blood samples was also done by using ADVIA 1800 which measures the turbidity produced by the anti-HbA1c antibodies.

Data analysis was carried out using computer software IBM SPSS (statistical package for social sciences) version 25. Quantitative variables including TAC, TOS, OSI ratio, FBG, and HbA1c were measured. TAC and TOS were expressed as mmol H_2O_2 Eqv./L, FBG as mg/dl, and HBA1c as mmol/mol (OSI being ratio has no specific units). The independent sample student t-test was applied for the comparison between the groups. A p-value ≤0.05 was considered significant.

Table I: Comparison of FBG (fasting blood glucose) and HbA1c levels between group I and group II.

Blood parameters	Group I Mean ± SD	Group II Mean ± SD	p-value
FBG (mg/dl)	91.61 ± 5.71	109.18 ± 12.32	0.001
HbA1c (mmol/mol)	5.43 ± 0.57	6.46 ± 0.85	0.001

Table II: Comparison of TAC, TOS, and OSI in the aqueous humour of cataract patients in group I and group II.

Aqueous humour parameters	Group I Mean ± SD	Group II Mean ± SD	p-value
TAC (mmol H_2O_2 Eqv./L)	2.62 ± 0.45	1.86 ± 0.41	0.001
TOS (mmol H_2O_2 Eqv./L)	33.75 ± 4.28	57.57 ± 5.46	0.001
OSI (arbitrary units) = [TOS (mol H_2O_2 Eqv./L)/ TAS (mmol Trolox Eqv./L)]	13.28 ± 3.02	32.79 ± 9.23	0.001

RESULTS

Independent sample student t-test was applied to compare the means. Both groups were found to be significantly different in all the above parameters as shown in Table I and II. FBG and HBA1c were significantly raised in the plasma of the diabetic group. In the aqueous humour, TOS was significantly high while TAC was

significantly low in the diabetic group. This resulted in significantly high OSI in the diabetic group. The power of the study was found to be >0.80.

DISCUSSION

Patients with diabetic cataract were found to have increased levels of HbA1c and FBG than the non-diabetic cataract group in this study. This suggests increase in the plasma glycemic index of the diabetic group, which is known to cause increase in the plasma oxidative stress.[12] Aqueous humour is a clear fluid present in the anterior and posterior ocular chambers which is derived from the plasma. It is formed by the ciliary processes which utilise the plasma to form the aqueous humour.[13] However, the chemical composition of aqueous humour differs from that of the plasma. Especially, there is a remarkable difference of proteins and amino acid concentrations between the two fluids. The protein concentration in the plasma ranges from 60 to 70g/L while in aqueous humour it ranges from 0.05 to 0.15g/L, which is essential for maintaining the optical transparency. The changes occurring in the plasma are likely to affect the aqueous humour.[14] Association of many oxidative stress markers with T2DM has been studied. These studies have mostly focused on only one marker at a time, present in the plasma of the diabetic patients. However, markers of oxidative stress are considered to act together with synergistic effects.[15] Keeping in view, estimating the total oxidative stress is a better indicator of the level of damage being endured by the patient. Further, the oxidative stress in the aqueous humour is more relevant to the cataract development compared to plasma.

In order to ascertain these effects, the authors measured the TAC, TOS, and OSI levels in the aqueous humour. The present results demonstrate that T2DM raises the TOS and reduces the TAC levels in aqueous humour as compared to non-diabetic cataract patients. This implies that, instead of the cataracts themselves, T2DM mainly affects the TOS and TAC levels. Rise in oxidative stress markers has been shown by Hashim *et al.* in T2DM patients with cataracts along with reduced antioxidant markers, which is in line with this study.[16]

In this study, TOS and OSI were found to be significantly increased (p-value=0.001) in the aqueous humour of diabetic compared to non-diabetic group thereby suggesting that the amount of oxidative stress put on the lens is also increased in the diabetics. The higher oxidative stress in diabetic cataract patients can be attributed to the rise in glucose concentration of the aqueous humour which causes glycation of the lens proteins. This leads to the production of sorbitol (an advanced glycation end product).[17] Sorbitol accumulation leads to the osmotic stress which causes production of free oxygen radicals by the endoplasmic reticulum of the lens. Free oxygen radicals cause oxidation and disulfide bond formation of the lens proteins thereby promoting the chances of cataract development.[18] Variation in glucose levels induces free oxygen radicals' production through an unfolded response causing damage to the Na/K-ATPase pump in lens epithelium. Hence, there is disturbed ion balance along with a decrease in protein solubility in the lens fibers. The lipid peroxidation-induced diminished membrane stability also contributes to the cataract development.[19]

Reduced antioxidant capacity of aqueous humour in diabetics as shown in similar studies, suggests its role in the pathophysiology of cataracts.[10] Increase in the plasma glucose and HbA1c levels reduces the aqueous humour antioxidant level. Lamont's study has shown TAC levels to be positively correlated with uric acid, glutathione, albumin, and other antioxidants.[16] As TAC is an estimation of the combined capacity of all antioxidants in a biological sample, measuring TAC provides an overall antioxidant status of a sample, which includes the effects of known as well as unidentified antioxidants. The TAC was found to be decreased in the diabetic group because the antioxidants are being used up to counter the increasing amounts of oxidants that are being produced.[20]

The patients included in this study had varied distribution of disease and their compliance and mode of treatment could not be documented, hence their impact on the results of this study is unknown. It is suggested that the cataract development may be prevented or delayed by optimal blood glucose regulation which would keep the oxidative stress in the aqueous humour low and anti-oxidant levels comparable to normal. Nevertheless, additional studies are required to establish the relationship between glycemic control, oxidative stress, and cataract development.

CONCLUSION

Increased oxidative stress in the blood of T2DM patients with cataract leads to high level of oxidative stress and low total antioxidant capacity in the aqueous humour. This increased free radical generation and weak antioxidant protection in the aqueous humour plays a significant role in the cataract development. In addition, poor glycemic control and increased duration of T2DM may also adversely contribute to this imbalance.

ETHICAL APPROVAL:

The study protocol was approved by the institutional Ethical Review Committee (ERC/ID/206 dated 30 May 2022). The study was conducted in accordance with the principles of the Declaration of Helsinki.

PATIENTS' CONSENT:

Informed consent were obtained from the patients and/or their families/legal guardians to publish the data concerning their cases prior to publishing the data.

COMPETING INTEREST:

The authors declared no competing interest with respect to the authorship and publication of this article.

AUTHORS' CONTRIBUTION:

SBA: Design of work, critical analysis and interpretation of data, and drafting of the manuscript.
AF: Literature search, acquisition of data, and appropriate investigations.
TAJ: Conception of work, data collection, and critical revision.
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